

Appl. No.
Amdt. dated April 21, 2005
Preliminary Amendment

PATENT

Remarks/Arguments begin on page 10 of this paper.

Amendments to the Specification:

Please amend the title as follows:

--~~GENE EXPRESSED SPECIFICALLY~~ EXPRESSED IN POSTMITOTIC DOPAMINERGIC
NEURON PRECURSOR CELLS ~~DOPAMINE PRODUCING NEURON PRECURSOR CELLS
AFTER TERMINATION OF DIVISION--~~

Please amend the paragraph on page 1, line 36 through page 2, line 17, beginning,
" In order to resolve the ethical issues and shortage of supply, methods have been..." as follows:

--In order to resolve the ethical issues and shortage of supply, methods
have been proposed that use, for example, porcine cortex, stria, ~~and~~ or midbrain
cells (for example, Published Japanese Translation of International Publication
No. Hei 10-508487, Published Japanese Translation of International Publication
No. Hei 10-508488 or Published Japanese Translation of International Publication
No. Hei 10-509034). In these methods, a complex procedure that involves the
alteration of cell surface antigens (MHC class I antigens) is required. Therefore,
the use of an *in vitro* differentiation system to generate dopaminergic neurons
from non-neural cells such as embryonic stem (ES) cells and bone marrow
interstitial cells instead of cells derived from aborted fetuses, is considered
promising. The importance of regeneration therapy using ES cells or a patient's
own neural stem cells is likely to grow in the future. A method involving local
immunosuppression by simultaneously transplanting Sertoli's cells has been
proposed as a method of eliminating transplant rejection (Published Japanese
Translation of International Publication No. Hei 11-509170, Published Japanese
Translation of International Publication No. Hei 11-501818, Selawry and
Cameron (1993) Cell Transplant 2: 123-9). It is possible to obtain transplant cells
from relatives that have matching MHCs, bone marrow from other individuals,
bone marrow banks, or umbilical cord-blood banks. However, if it were possible

to use the patient's own cells, the problem of rejection reactions can be overcome without any laborious procedures and trouble.--

Please amend the paragraph on page 4, lines 5 through 22, beginning, " According to the above results, anti-65B13 antibodies can be used to obtain pure..." as follows:

--According to the above results, anti-65B13 antibodies can be used to obtain pure early-stage dopaminergic neuron precursor cells, by isolating 65B13-expressing cells from ventral midbrain region or cell cultures ~~culture media~~ that contain *in vitro*-differentiated dopaminergic neurons. Cells obtained in this manner contain only postmitotic precursor cells, and since only the cell type of interest is isolated, these cells are extremely safe even when used for transplant therapy. Since the earliest possible precursor cells are used, high therapeutic efficacy can be expected in terms of their survival rate, network formation ability, and such. Further, in the cases where the best therapeutic effects cannot be achieved by these early precursor cells obtained immediately after cell cycle exit, and where the use of matured cells is required, early precursor cells obtained by this method can simply be cultured *in vitro* to mature into a suitable stage of differentiation. Thus, materials that are in a differentiation stage suitable for the target transplant therapy can be easily prepared (Fig. 12).--

Please amend the paragraph on page 4, line 23 to page 5, line 28, beginning, "More specifically, the present invention relates to:..." as follows:

--More specifically, the present invention relates to:

[1] a polynucleotide that comprises a sequence selected from the nucleotide sequences of (1) to (5) ~~(4)~~, wherein the nucleotide sequences encode 65B13 polypeptide expressed specifically in dopaminergic neuron precursor cells immediately after cell cycle exit, or antigenic fragment thereof:

- (1) a nucleotide sequence that comprises the 177th to 2280th nucleotides of SEQ ID NO: 1 or the 127th to 2079th nucleotides of SEQ ID NO: 2, or sequence complementary to said nucleotide sequence;
- (2) a nucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 3 or 4, or sequence complementary to said nucleotide sequence;
- (3) a nucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 3 or 4, wherein a signal sequence portion is deleted, or sequence complementary to said nucleotide sequence;
- (4) a nucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 3 or 4, wherein one or more amino acids have been deleted, inserted, substituted, or added, or sequence complementary to said nucleotide sequence; and,
- (5) a nucleotide sequence that hybridizes with the nucleotide sequence (1) under stringent conditions;
 - [2] a vector that comprises the polynucleotide of [1];
 - [3] a host cell that comprises the polynucleotide of [1] or the vector of [2];
 - [4] a polypeptide that is encoded by the polynucleotide of [1];
 - [5] a fragment of the polypeptide of [4], wherein the polypeptide fragment comprises at least eight amino acid residues;
 - [6] an antibody against the polypeptide of [4] or the polypeptide fragment of [5];
 - [7] a nucleotide chain that encodes the polypeptide fragment of [5];
 - [8] a method for selecting a dopaminergic neuron, wherein the method comprises the step of contacting the antibody of [6] with a cell sample thought to comprise a dopaminergic neuron precursor cell;
 - [9] a method for selecting a dopaminergic neuron, wherein the method comprises the step of contacting a peptide comprising at least the extracellular portion of the polypeptide of [4] with a cell sample thought to comprise a dopaminergic neuron precursor cell;

[10] a Dopaminergic neuron precursor cell immediately after cell cycle exit, wherein the cell is selected by the method of [8] or [9];

[11] a method for isolating a gene specific to a dopaminergic neuron precursor cell, and a gene specific to each stage of maturation into a dopaminergic neurons, wherein the method comprises the step of: detecting and isolating a gene specifically expressed in the precursor cell of [10] or a cell differentiated, induced, or proliferated from said precursor cell; and

[12] a method for screening using maturation as an indicator, wherein the method comprises the steps of: contacting a test substance with the precursor cell of [10]; and detecting the differentiation or proliferation of the precursor cell resulting from the contacting step.--

Please amend the paragraph on page 25, line 30 through page 26, line 1, beginning, "In addition, dopaminergic neuron precursor cells can also be selected using a..." as follows:

--In addition, dopaminergic neuron precursor cells can also be selected using a promoter for 65B13 (see, for example, Unexamined Published Japanese Patent Application No. 2002-51775). For example, a vector harboring a construct that comprises a gene encoding a detection marker, such as GFP, linked to a promoter region obtained from analyzing the 65B13 expression regulatory regions to be described later, can be transfected into cells. In addition, a gene encoding a marker can also be knocked in at the 65B13 gene locus. In either case, specific cells can be selected by detecting the expression of a marker gene specific for dopaminergic neuron precursor cells.--

Please amend the paragraph on page 26, lines 2 through 19, beginning, "The cell sample used here preferably comprises cells of the ventral midbrain region or..." as follows:

--The cell sample used here preferably comprises cells of the ventral midbrain region or cell culture ~~culture-medium~~ containing *in vitro* differentiated

dopaminergic neurons. *In vitro* differentiation of dopaminergic neurons can be carried out by known methods using cells such as known ES cells, bone marrow interstitial cells, immortalized neuron-derived cell lines (Published Japanese Translation of International Publication No. Hei 8-509215; Published Japanese Translation of International Publication No. Hei 11-506930; Published Japanese Translation of International Publication No. 2002-522070), or primordial neuron cells (Published Japanese Translation of International Publication No. Hei 11-509729), as the starting material. Normally, dopaminergic neurons can be differentiated by co-culturing a tissue obtained from a dopaminergic neuron region of the brain, with a sustentacular cell layer derived from neural tissues. Moreover, methods are also known for deriving dopaminergic cells from neural tissues that normally do not produce dopamine, such as the striatum and cortex (Published Japanese Translation of International Publication No. Hei 10-509319). In addition, culturing under hypoxic conditions has been reported to produce cells containing a greater number of dopaminergic neurons (Published Japanese Translation of International Publication No. 2002-530068). A cell sample used in the selection of dopaminergic neuron precursor cells of the present invention may be a cell population isolated or cultured by any method.--

Please amend the paragraph on page 26, line 31 through page 27, line 9, beginning, "Since cells obtained in this manner are postmitotic neuron precursor cells, they are..." as follows:

--Since cells obtained in this manner are postmitotic neuron precursor cells, they are preferable in transplant therapy for neurodegenerative diseases, such as Parkinson's disease, in terms of their safety, survival rate, and network formation ability, compared to conventional mixed cell populations or dopaminergic neurons carrying an exogenous gene. Moreover, since cells (or cell populations) of the present invention obtained according to the methods of this invention are immediate postmitotic precursor cells, they can also be

differentiated into a suitable stage by selecting *in vitro* conditions such as media, and are preferable materials for various types of neural transplant therapy. When neuron precursor cells obtained using the methods of the present invention are used in transplants, preferably 1×10^3 to 1×10^6 cells ~~neurons~~, and more preferably 5×10^4 to 6×10^4 cells ~~neurons~~, are transplanted. The primary method is stereotaxic surgery in which a cell suspension is transplanted into the brain. In addition, cells may also be transplanted by microsurgery. See, Backlund et al. (Backlund et al. (1985) J. Neurosurg. 62: 169-73), Lindvall et al. (Lindvall et al. (1987) Ann. Neurol. 22: 457-68) or Madrazo et al. (Madrazo et al. (1987) New Engl. J. Med. 316: 831-4), for methods of transplanting neuron tissues.--

Please amend the heading on page 31, line 11 as follows:

--<Analysis of 65B13 Expression Regulatory Region>--.

Please amend the paragraph on page 31, line 36 through page 32, line 2, beginning, "The expression region of the 65B13 gene isolated in this manner can be used to..." as follows:

--The expression regulatory region of the 65B13 gene isolated in this manner can be used to produce a protein of interest specific for postmitotic dopaminergic neuron precursor cells *in vivo*.--

Please amend the paragraph on page 37, lines 19 through 35, beginning, " The nucleotide sequences of the resulting two genes of 65B13-a and 65B13-b are..." as follows:

-- The nucleotide sequences of the resulting two genes of 65B13-a and 65B13-b are shown as SEQ ID NO: 1 (Figs. 1 and 2) and SEQ ID NO: 2 (Figs. 3 and 4). The coding region of 65B13-a begins at the 177th "A" of SEQ ID NO: 1 and ends with the stop codon at nucleotides 2278 to 2280, yielding a protein comprising 700 amino acids. The 17 amino acid residues encoded by the sequence of nucleotides 177 to 228 is the signal sequence. The 17 amino acid

residues encoded by the sequence of nucleotides 1717 to 1767 is the transmembrane domain. In contrast, the coding region of 65B13-b begins at the 127th "A" of SEQ ID NO: 2 and ends at the stop codon of nucleotides 2077 ~~2277~~ to 2079, yielding a protein comprising 650 amino acids. The 17 amino acid residues encoded by the sequence of nucleotides 127 to 177 is the signal sequence, and the 17 amino acid residues encoded by the sequence of nucleotides 1516 to 1566 is the transmembrane domain. The amino acid sequences encoded by the 65B13-a and 65B13-b genes are shown in SEQ ID NOs: 3 and 4. As shown in Fig. 5, a comparison of the amino acid sequences encoded by both genes revealed that 65B13-a and 65B13-b are isoforms that have resulted from alternative splicing, and that 65B13-b lacks 50 amino acids at the N-terminus compared to 65B13-a. Based on the homology search results, the proteins encoded by the 65B13 genes are believed to be single transmembrane proteins with five Ig domains as shown in Fig. 6.--

Please cancel the present "SEQUENCE LISTING", pages 1/39 - 39-39, and insert therefor the accompanying paper copy of the Substitute Sequence Listing, page numbers 1 to 14, at the end of the application.